The perfect stage of an isolate of *Rhizoctonia solani* from kenaf[1]

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Abstact: A kenaf isolate of *Rhizoctonia solani* was successfully induced to produce basidial state with PYDA-clay soil method *in vitro*. Basidia were 8.7—(11.9)—17.5 ×6.1—(7.6)—10.0 μ, sterigmata were almost the same length as basidia, and basidiospores measured 5.5—(7.6)—10.0×3.7—(4.9)—6.3 μ. The perfect stage of the organism was identified to be *Thanatephorus cucumeris* (Frank) Donk.

Although *Rhizoctonia solani* Kühn is an important soil-borne plant pathogen, the taxonomic position of the perfect stage has been a matter of some speculation. It has been described as a species of *Hypochnus*, *Botryobasidium*, *Pellicularia*, *Ceratobasidium* or *Thanatephorus* by various investigators[1,2,5,7,8,9]. This taxonomic confusion is mainly due to the infrequency of basidial production of the organism.

R. solani has long been known to cause damping-off on kenaf[11]. Although the vegetative cell of the kenaf-isolate of *R. solani* is known to be multinucleate[12], the basidial state of the isolate has not been described. This study presented a method for inducing the formation of basidial stage of the kenaf-isolate of *R. solani*. The somatic features and the basidial state of the organism were also described so that the taxonomic position of the organism could be determined.

Materials and Methods

R. solani isolate K-1 was originally isolated from diseased mature kenaf in 1968. A stock culture of the fungus was maintained in the laboratory by frequent transfers to fresh potato-sucrose agar (PSA).

A modified method of the fructification on soil surface reported by Stretton *et al*[10] was adapted. The fungus was grown on potato yeast extract dextrose agar (PYDA) in 9-cm Petri dish for 4 to 7 days at 28°C. The lid was then removed, culture was covered with 1 cm deep sterilized Kuang-miao hillside clay soil (approximately 63.59 cm^2 culture with 94.8 g of dry weight soil), moistened 1 to 3 times each day, and incubated at 25–28°C under continuous light (200 ft-c). Fructification occured with uniform success after 3–14 days. The fruiting body appeared on the surface of soil as a whitish grey thin layer. Fourteen days after fruiting, all basidia were collapsed and no more basidium could be found. The old hymenium layer, at this time, turned to yellowish white-grey in color.

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The Kuang-miao hillside clay soil mentioned above served as a medium for basidial formation of the kenaf-isolate of *R. solani*. The soil was obtained from the hillside located in Kuang-miao village of Tainan Prefcture. The soil was first air-dried, and passed through a 25 mesh seive. The fine soil collected was autoclaved with 15 lb for 30 min.

For the observation of the somatic features of the kenaf-isolate of *R. solani*, the fungus was allowed to grow on PSA at 28°C for 2 and 18 days, respectively. Small pieces of the colonies were removed carefully and observed under microscope. The method for nuclear count of the hyphal cell was based on that reported by Tu *et al*.[13]. Mycelium to be stained was grown on PSA for 10 days. A small piece of mycelial mat was removed and fixed in modified Carnoy's Fixative for 18–24 hr. Fixed mycelial mats were washed in 50% acetic acid, hydrolyzed in 0.1 N HCl solution at 65°C for 20–30 min., and rinsed again in 50% acetic acid. They were stained with 2% Orcein in 50% acetic acid for 6–8 hr. The nuclei hyphal tip cells and in the other cells were counted.

Results

Somatic features of the kenaf-isolate of R. solani: The asexual features of the orgaism show the typical characteristics of *R. solani*. The vegetative hyphae are septate, hyaline at first, later becoming cinnamon-brown. The most mature hyphae are dark brown, rather rigid, the cell wall thicker, with some cruciform cells eventually developing into brown sclerotia. The hyphal branches are inclined in the direction of growth when young. They assure a right angle to the main hyphae, and constrict at the branchebabase (Fig. 3). The first septum in the branch is formed at 6.1–(9.5)–16.2 μ from the point of origin. The hyphae are devoid of clamp connections. The width of main hyphae is 5.0–(6.6)–12.0 μ, and that of branches is 5.0–(7.0)–13.8μ. The cruciform cells, also called chlamydospores or monilioid cells, form in chain (Fig. 4.). The size of cruciform cell is about 20.0–(24.4)–32.5×10.0–(12.9)–17.5 μ.

Sclerotia are formed abundantly in the older colonies on the surface of PSA, frequently imbedded in the mycelium and bound together by mycelial bands. The sclerotia are brown, globose, more or less flattened, and are not differentiated into rind and medulla in structure (Fig. 1,2).

The hyphal cells are multinucleate (Fig. 5, 6, 7). The number of nuclei varies widely from cell to cell, but is only slighly different between tip and other cells. In the tip cells, the range is from 2 to 8 nuclei, in other cells, 4 to 9. In the majority of the hyphal tip cells, however, the range is 5 to 6 nuclei, and in the cells other than tip cells, 5 to 8. The size of the nucleus is approximately 1.7–2.3 μ. Numbers of nuclei in growing tip and in other cells of mycelia are shown in Table 1.

Table 1. Distribution of nuclei in hyphal cells of the kenaf-isolate of *Rhizoctonia solani*

Number of nuclei per cell	Frequency or number of cells containing the giving number of nuclei	
	Tip cell	Other cell
2	2	0
3	2	0
4	7	13
5	15	30
6	21	61
7	12	24
8	5	18
9	0	1
Total	64	147

The basidial stage of the kenaf-isolate of R. solani: The fruiting body of the kenaf-isolate of *R. solani* was easy to recognize without magnification. It appeared on the surface of soil as a whitish grey thin layer (Fig. 8). Under the microscope, the fructification was seen to be resupinate, delicate, thin, white, purinose or floccose or arachnoid, with a discontinuous hymenium formed of clusters of basidia (Fig. 9). However, the subhymenium is usually divided cymosely, several times bearing the terminal basidia.

Basidia are subcylindric and barrel-shaped, or obpyriform or clavate, 8.7–(11.9)–17.5×6.1–(7.6)–10.0 μ, without median constriction or stipitate base, and about 1.5 times wider than the supporting cell. Sterigmata, 2-4, mostly 4, per basidium, are stout, more or less straight and slightly divergent, not curved like horns, 6.3–(10.3)–16.2 μ long and 1.2–(2.4)–3.2 μ wide at the base (Fig. 10, 11, 12, 13). Basidiospores measured 5.5-(7.6)-10.0×3.7–(4.9)–6.3 μ, and are hyaline, not amyloid, smooth, thin-walled, oblong to ellipsoid with one side flattened, or broad ovoid with a truncate spiculus, germinating by repetition (Fig. 14, 15). All basidia collapsed after the discharge of basidiospores. The detailed measurement of basidial structures are shown in Table 2.

Table 2. The measurement (μ) of basidial structures of the kenaf isolate of *Rhizoctonia solani**

Basidial structure	Length			Width		
	Range (μ)	Mean (μ)	C.V. (%)	Range (μ)	Mean (μ)	C.V. (%)
Basidia	8.7–17.5	11.9±1.5	12.0	6.1–10.0	7.6±0.4	5.9
Sterigmata	6.3–16.2	10.3±2.0	19.0	1.2– 3.2	2.4±0.2	10.0
Spores	5.5–10.0	7.6±0.8	11.0	3.7– 6.3	4.9±0.4	8.8

* based on 400 samples.

Discussion

Since Flentje[4] successfully induced the basidial formation of *R. solani* on soil surface *in vitro*, his method has been widely utilized and provided with great advantages in clarifying the taxonomic confusion of *R. solani* and the related fungi. The PYDA-clay soil method reported in this study gave a satisfactory result of inducing basidial formation of the kenaf-isolate of *R. solani*. Various kinds of soil, including sand, sandy loam, and clays collected from different places failed to obtain basidial formation, except the clay collected from hillside located in Kuang-miao village of Tainan Prefecture. Reasons of this success are waiting for further clarification.

Parmer and Whitney[6] suggested that *R. solani* is typified by (i) multinucleate cells in young vegetative hyphae, (ii) a prominent septal pore apparatus, (iii) branching near the distal septum in young vegetative hyphae, (iv) constriction of the branch base, (v) formation of a septum in the branch near the point of origin, and (vi) some shade of brown pigmentation. Our fungus showed the typical characteristics of *R. solani* as Parmeter and Whitney mentioned, except that the septal pore apparatus was not observed. The features mentioned above, together with the monilioid cells, undifferentiated sclerotia and lack of clamp connections, indicating that our fungus is a typical isolate of *R. solani*.

The basidial structures of our fungus also showed typical characteristics of *Thanatephorus cucumeris* (Frank) Donk[14]. The measurements of basidia, stergmata, and basidiospores were similar to those reported by Rogers[8] and Warcup and Talbot[14], but do not fall into any form species as Exner[3] reported. Depending upon the morphology of basidial structures alone will not provide enough information for grouping the fungi among the species. Anastomosis is the most dependable single criterion for determining group alliance as suggested[6].

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Rhizoctonia solani 鐘麻菌株之有性世代

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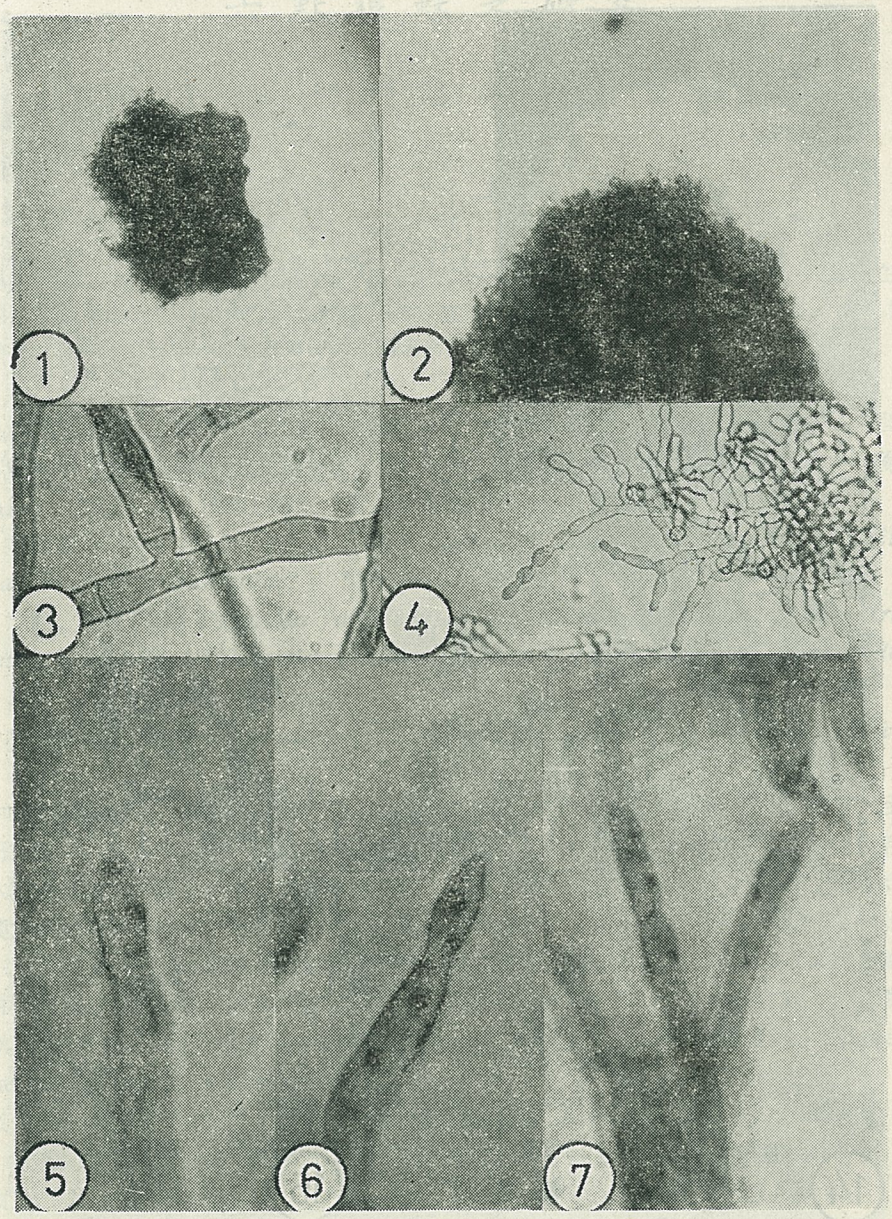
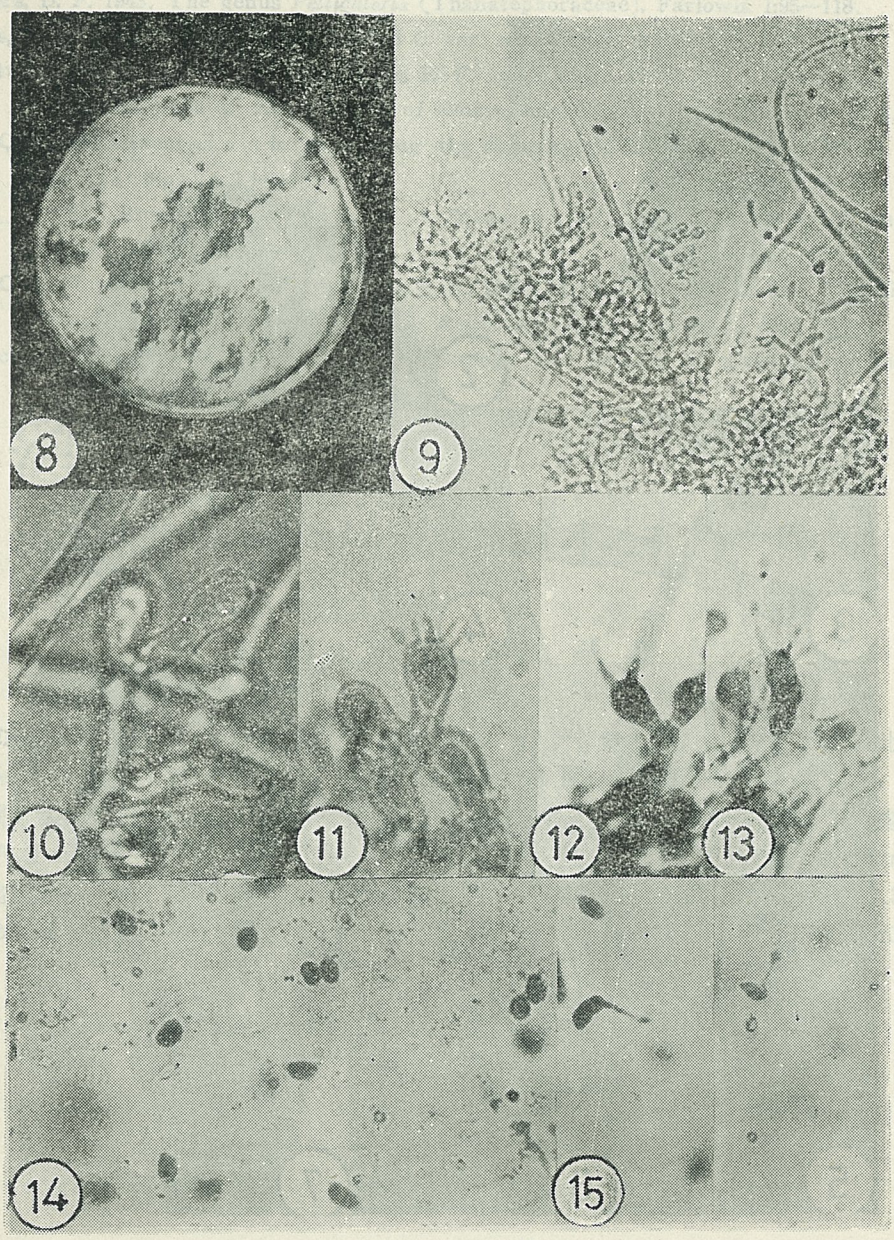
Rhizoctonia solani 是一種重要之土壤傳播病原菌，加害鐘麻引起嚴重之腰折病。此一菌株 ***R. solani*** K-1 利用 PYDA (Potato Yeast Extract Dextrose Agar) 培養4～7天後，蓋覆自臺南縣關廟鄉山坡掘取之粘土，調整適當濕度後經6天即可誘生有性世代。其有性世代（即擔子世代）之擔子 (Basidia) 大小爲 8.7—(11.9)—17.5×6.1—(7.6)—10.0 μ，擔孢子柄 (sterigmata) 之長度略同於擔子者，而擔孢子 (Basidiospore) 爲 5.5—(7.6)—10.0×3.7—(4.9)—6.3 μ。此一 ***R. solani*** 鐘麻菌株有性世代經鑑定認爲是 ***Thanatephorus cucumeris*** (Frank) Donk。



Figs. 1-7. Somatic features of *Thanatephorus cucumeris* (Frank) Donk (= *Rhizoctonia solani* Kühn) isolate K-1. 1. Sclerotium, x100. 2. A close view of sclerotial surface, x150. 3. Hyphal branch, x500. 4. Monilioid cell, x150. 5. Binucleate hyphal cell, x1,200. 6. Multinucleate (4 nuclei) hyphal cell, x1,200. 7. Multinucltinu hyphal cells containg 7 nuclei (left) and 5 nuclei (righ), x800.



Figs. 8-15. Basidial structures of *Thanatephorus cucumeris* (Frank) Donk (= *Rhizoctonia solani* Kühn) isolate K-1. 8. Fructification on soil surface. 9. Hymenium layer and basidial branches, x150. 10 Young basidia, x1,000. 11. Young basidia with 3 young sterigmata (epibasidium), x1,000. 12. A mature basidium with 2 sterigmata and a basidiospore, x700. 13 A mature basidium with 4 sterigmata and 4 young basidiospores, x650. 14. Mature basidiospores, x600. 15. Basidiospore germination by repetition x600.